

MORPHOLOGY AND PHYSIOLOGY OF THE MAUTHNER CELL IN COD, *GADUS MORHUA*

BAO-QUEY HUANG¹ and PETER J. FRASER²

Department of Fisheries, National Taiwan Ocean University,
Keelung, Taiwan 20224, Republic of China¹

and

Department of Zoology, Aberdeen University,
Aberdeen, AB9 2TN, Scotland, U.K.²

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Bao-Quey Huang and Peter J. Fraser (1991) Morphology and physiology of the Mauthner cell in cod, *Gadus morhua*. Bull. Inst. Zool., Academia Sinica 30(3): 227-236. Morphological and electrophysiological studies on the Mauthner cells of cod (*Gadus morhua*) were conducted. The results demonstrated that the teleost has fulfilled all four morphological criteria for Mauthner cells. These include conspicuous soma at the level of the 8th cranial nerve, two distinctive dendrites (lateral and ventral) a large axon, and a specific axon cap. A conspicuous ventro-lateral dendrite extended from the soma and branched distally was found in cod. The extracellular antidromic Mauthner cell response is a short latency (1.9 msec), with an all or none negative potential, and characteristically fits the physiological criteria of Mauthner cells.

Key words: Mauthner cells, Morphological criteria, Electrophysiological criteria, Cod.

The Mauthner system has close links with a characteristic startle or fast start response, indicated by a perfect time correlation between the initiation of the Mauthner spike and the start of a behavior response (Webb, 1978; Eaton *et al.*, 1981, 1982). The timing of their activity relative to the behavior response agrees well with the hypothesis that the Mauthner cell triggers the observed motor pattern, and the cell never fires after the startle behavior has commenced (Faber and Korn, 1978; Eaton, 1983). Furthermore, the Mauthner impulse is sufficient to evoke a short latency all or none electromyographic response that corresponds temporarily with this behavior (Hackett and Faber, cited in Eaton, 1983). This evidence suggests that the Mauthner cell

plays a role as a command neurone in the startle response (Eaton, 1983).

Mauthner cells have long been known to be located at the level of the auditory nerve (the 8th cranial nerve) in the medulla oblongata of many lower aquatic vertebrates, elasmobranchs, agnatha, teleosts and some amphibians (Retzlaff, 1957; Eaton *et al.*, 1977; Faber and Korn, 1978). Of the 20,000 species of living teleosts, Mauthner cells have been investigated at the level of electrophysiology and behavior only in a few species, mostly cypriids (Kimmel *et al.*, 1980; Zottoli, 1981; Eaton *et al.*, 1981, 1982). The Mauthner cells of more than 200 species have been studied morphologically to date, and the data has revealed significant differences in size, disposition of the dendrites, and the structure of their axon and axon cap

(Zottoli, 1978a; Faber and Korn, 1978). These specific differences suggest the inadequacy of generalizations regarding the physiological and ethological characteristics of the Mauthner cells.

The cod Mauthner cell has not been investigated previously except for in a study conducted by Zottoli (1978a) which measured the size of the Mauthner cells in one individual cod. It is, however, conventional that several morphological and electrophysiological criteria are used for identification and comparative studies of Mauthner cells. Four morphological criteria are used in the majority of teleosts including: (1) A large soma located at the level of the 8th cranial nerve; (2) Two main dendrites located laterally and ventrally; (3) The axon, the largest axon in the spinal cord; and (4) The axon cap, a particular neuropile surrounding the unmyelinated area of its axon and axon hillock (Faber and Korn, 1978). In order to further confirm the identification, three electrophysiological criteria were used. These included: (1) The antidromic extracellular potential has a short latency and is an all or none spike; (2) Either antidromic or orthodromic activations could elicit a characteristically large amplitude negative extracellular potential; (3) The amplitude of the action potentials vary with the recording site on the recording cell (Zottoli, 1977; Eaton *et al.*, 1977; Eaton 1983). However, a general description of Mauthner cells, a basis for their identification, and a characterization of their morphology and physiology will be discussed in the present studies.

MATERIALS AND METHODS

Cod, *Gadus morhua*, with a body length ranging from 25 to 40 cm were obtained locally from the North Sea around Aberdeen and acclimated in hold-

ing tanks for at least two days. Fish were anaesthetized by an intraperitoneal injection of Saffan (Alphaxalone Alpha-dolone acetate 3:1) at the dosage of 12-18 mg/kg body weight (Horner, 1980). By a single injection of Saffan, the fish normally remained anaesthetized for the experiment duration. To calculate the dose required without unnecessary handling of the experimental fish, the weight was calculated from the length of the fish using the linear body weight-length relationship for lengths ranging between 20 and 40 cm (Fig. 1). Transverse, sagittal and horizontal serial sections of cod brain were obtained following perfusion of the circulatory system with Duboscq-brasil fixative, wax embedding and sectioning at 10 μ m. These were stained using the Holmes silver method which employs pyridine as a mordant and stains both cells and fibers (Guthrie, 1980).

For electrophysiological recordings, the fish were anaesthetized as above for 30 minutes when the opercular movements had slowed down, the fish was quickly clamped into the experimental tank and fixed with a pair of eye bars and a nose bar to immobilize the head (Fig. 2). A further clamp supported the body of the fish. During experiments aerated and filtered seawater was supplied continuously to fish buccopharynx *via* a hard mouth tube. This hard tube was used in conjunction with the nose bar to stabilize the head. The water flow rate through the branchial chamber was maintained approximately 1,500 ml/min. When the fish were fully anaesthetized a mid-cranial incision was made with a scalpel and the cut was continued on one side of the dorsal ridge. In order to expose the cranial bone, overlying muscles were carefully removed to avoid any damage to blood vessels underneath. The optic tectum and cerebellum were revealed by removing the cranial bone and the

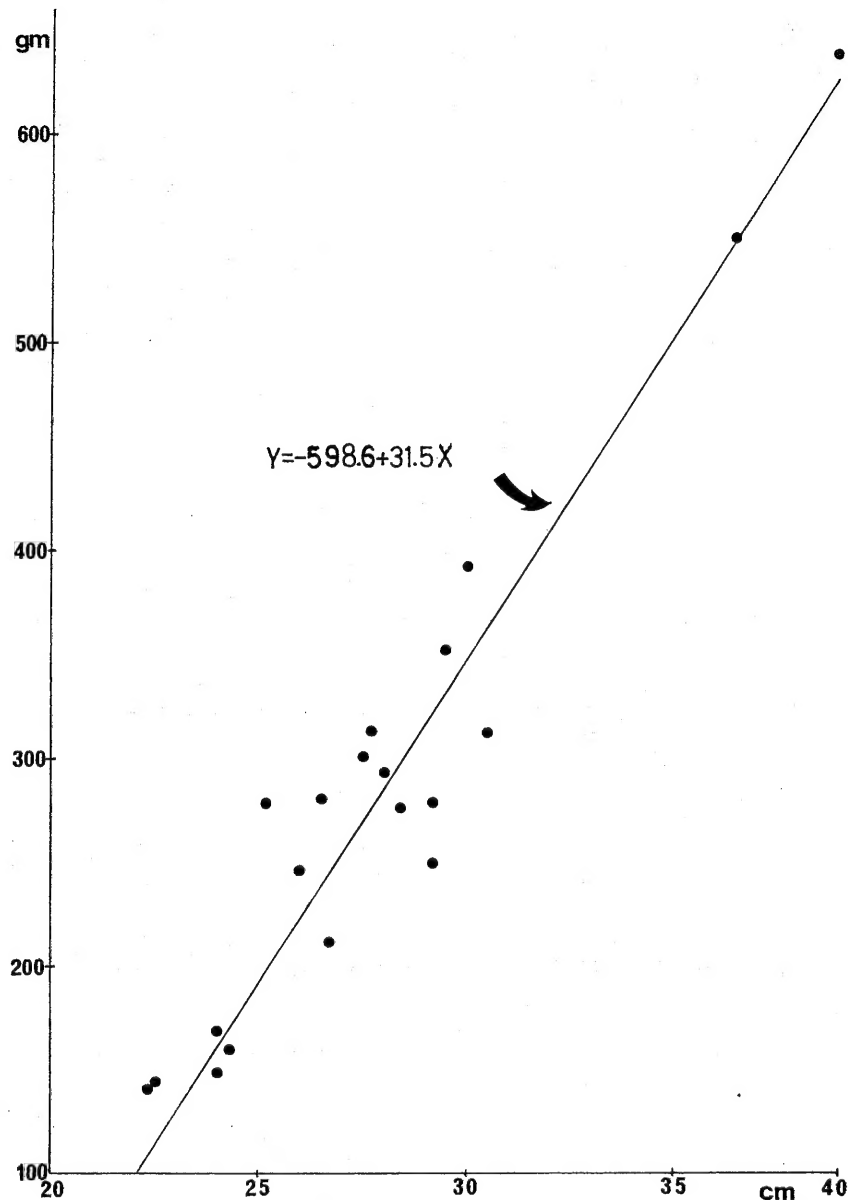


Fig. 1. Relationship between body weight (gm) and body length (cm) of cod for anaesthetic administration.

meninges in such a way as to minimize bleeding.

Recording electrodes were made from 1.5 mm diameter capillary tubing containing fibres to aid filling (Clark Electromedical GC150F-15). Electrodes were pulled by a vertical puller (Bioscience Microelectrode Puller) and filled fitted with a membrane filter (Sartorius, GMBH,

pore size $0.45 \mu\text{m}$). An electrical connection was made with a silver wire which was chlorided and soldered to a 1 mm plug to minimize junction potentials (Purves, 1981; Brown and Fyffe, 1984). A NL 102 high impedance amplifier with a NL 100 headstage was used to interface the recording electrode to an oscilloscope. The electrode impedance was measured using

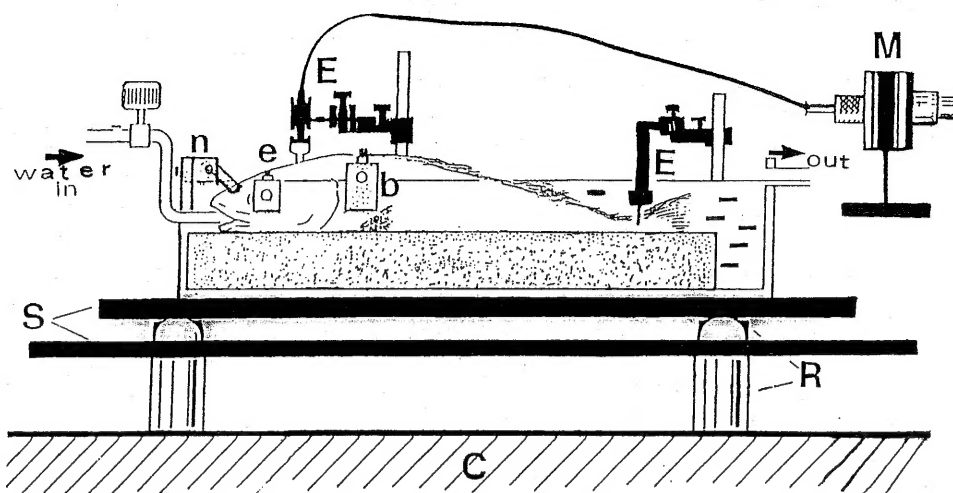


Fig. 2. The experimental set-up for the Mauthner cell recording. Note the nose (n) and ear (e) bars, body clamps (b) for firmly holding the anaesthetized cod. E: electrode holders, M: hydraulic micromanipulator, S: metal plate, R: rubber pads, C: concrete table.

an inbuilt electrode test facility (Huang, 1986). Electrodes were used with impedances between 15 and 30 M for an extracellular recording and between 30 and 40 M for an intracellular recording. The microelectrode on the headstage was fixed to a one dimensional hydraulic micromanipulator (Narishige Scientific Instrument MO-8), which in turn was mounted on a Prior manipulator allowing control over three axes of displacement. Bipolar stimulating electrodes were placed on the exposed vertebral column near the caudal base of the dorsal fin about 20 cm from the recording site to activate the Mauthner axon antidromically. A Farnell stimulator was used to produce stimulating pulses of 0.3–1 msec duration, 0–100 volts amplitude at frequencies of 0.5–10 Hz. The microelectrode was lowered to a point 0.5–0.8 mm posterior to the optic tectum at the level of the 8th cranial nerve and 0.5 mm to either side of the midline.

Electrically evoked responses could be monitored on the oscilloscope and stored in an FM tape recorder (Tandberg Instrumentation Recorder, Series 115) for later analysis. The response could also be averaged using a NL 750 signal aver-

ager triggered from a synchronizing pulse from the Farnell stimulator *via* a NL 200 spike trigger, and plotted on a pen recorder at slow speed. The fish holder, electrode carriers and the micromanipulators were mounted on an earthed plate mounted *via* rubber shock absorbers on a heavy concrete table. The whole set-up was shielded by a surrounding Faraday cage (Huang, 1986).

RESULTS AND DISCUSSIONS

I. Histology

The two Mauthner cells, easily identified in the cod, correspond in morphological terms to those of other species such as goldfish (Nakajima, 1974; Zottoli, 1977; Zottoli *et al.*, 1984), bullhead (Retzlaff, 1957), Atlantic tomcod, (Zottoli, 1978b) and winter flounder (Zottoli, 1981). Mauthner cells in cod show all four morphological criteria: conspicuous soma at the level of the 8th cranial nerve, two distinctive dendrites (lateral and ventral), large axon and specific axon cap.

The cod's Mauthner cell has a conspicuously large soma whose position at the level of the 8th cranial nerve in the

medulla oblongata is shown in transverse sections (Fig. 3). The soma diameter was about $60\text{ }\mu\text{m}$ and the cell area was about $3,000\text{ }\mu\text{m}^2$. Holms stained serial sections also revealed various nuclei and fiber tracts in relation to the Mauthner cell in the cod. The ventral dendrites approach the nucleus motorius tegmenti and the tectobulbar tracts, which are known to send efferent fibers from the optic tectum to the lower brain. The Mauthner cell is located approximately $400\text{--}500\text{ }\mu\text{m}$ to the side of the midline and at a depth of about $4.5\text{--}5\text{ mm}$ from the surface of the brain (Fig. 3). The disposition of axon, and the lateral and ventral dendrites are detailed in Figs. 4A and 4B. In addition, these figures show a conspicuous ventro-lateral dendrite with a diameter of about $5\text{ }\mu\text{m}$ extended from the ventro-lateral corner of the soma and branched distally. The differences between the endings on the soma and on the dendrites are not clearly distinguishable. In the proximal part of the lateral dendrite and also in the soma apart from the axon hillock, the pattern of branches is consistent with a dense covering of synaptic endings (Fig. 4). Some of the endings appear to be large myelinated club endings as in goldfish (Zottoli, 1978b). Under the light microscope, these endings have large diameters and lose their myelin abruptly when approaching the Mauthner cell dendrites so that they form blunt ends.

The axon of the cod Mauthner cell is large compared with surrounding fibers, with a diameter of about $15\text{ to }20\text{ }\mu$. The axons run medially, somewhat anteriorly and then cross the midline to the contralateral side of the spinal cord. The axon cap is visible as a characteristic neuropile which is divided into two parts. The central core consists of the axon hillock (Fig. 4C), the initial segment and the surrounding fine spiral fibers. These form

a helicoidal network around the initial segment and are thought to be from the supracommissural bundle of mesencephalic nucleus of the Fasciculus longitudinalis medialis (Zottoli, 1978). The peripheral region consists of glial cells, and large axonal processes (Fig. 4C). The glial cells, which are known as the cap wall, can be seen as a wall like layer around the axon cap (Fig. 4C). A montage of the cell reconstructed from transverse sections is shown in Fig. 4D.

Teleosts are known to have two morphologically distinct types of axon caps to the Mauthner cells, and the cap structure is family-specific (Ostuka, 1964). Type I is characterized by a layer of glial cells surrounding the spiral fibers, which are wrapped around the initial segments of the Mauthner axon, and is commonly found in cyprinids. Type II axon cap does not have glial cells surrounding the spiral fibers, and is represented by flounders. The axon cap of the cod shows a layer of glial cells hence placing cod Mauthner axons into type I. This further fits with the placing on size criteria and supports the assumption that the cod has a functional Mauthner mediated startle response (Huang and Fraser, 1989).

II. Antidromic Mauthner cell activation

The Mauthner cell was identified, after antidromic stimulation, by a distinctive extracellular spike which was thought to be generated at the initial segment and axon hillock region (Eaton *et al.*, 1977, 1981). It has a characteristically short latency which could be distinguished from the neighboring slower conducting neurones. With a stimulating site about 20 cm posterior to the recording site, the latency was $1.9\pm 0.5\text{ msec}$ (Mean \pm standard deviation, $n=11$) (Fig. 5).

The extracellular antidromic Mauthner cell response recorded from the cod,



Fig. 3. Photomicrograph of the paired Mauthner cells and their related structures. VIII. 8th cranial nerve, VI: 6th cranial nerve, IV: 4th ventricle, NMT: nucleus motorius tegmenti, TB: tectobulbar tract, FLM: fasciculus longitudinalis medialis. Black bar indicates 1 mm.

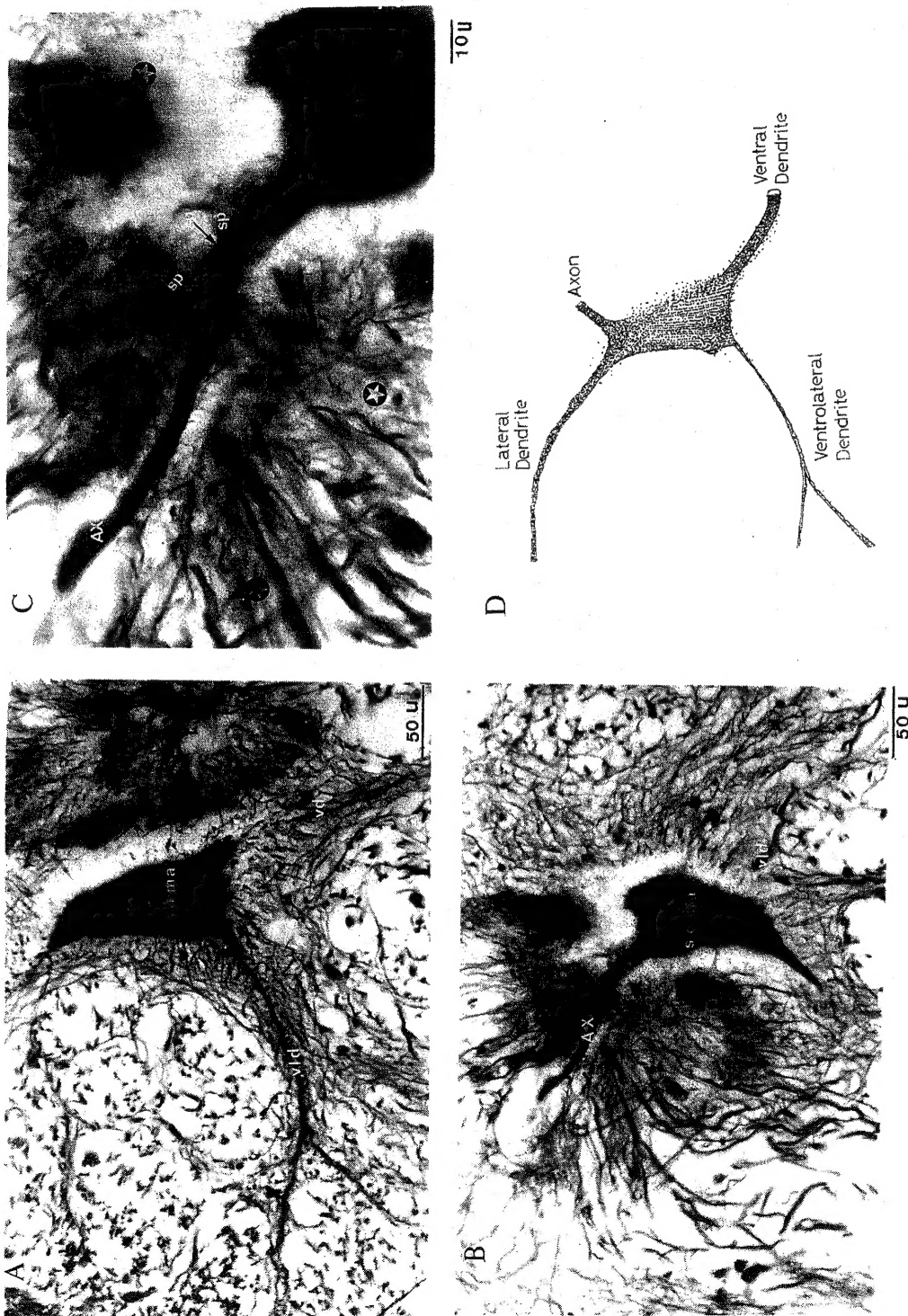


Fig. 4. Histological sections and a reconstructed drawing of the Mauthner cells.

A: A left Mauthner cell revealed a clear ventro-lateral dendrite (vld.)

B: A right Mauthner cell revealed a clear axon (AX) and ventrolateral dendrite (vld.).

C: Axon cap and peripellular net. Note the central core (▷◁) and peripheral portion (☆).

Central core has thin fibers spiral (sp) courses around the axon and peripheral area is surrounded by a cap of glial cells.

D: A montage of the Mauthner cell reconstructed from several transverse sections.

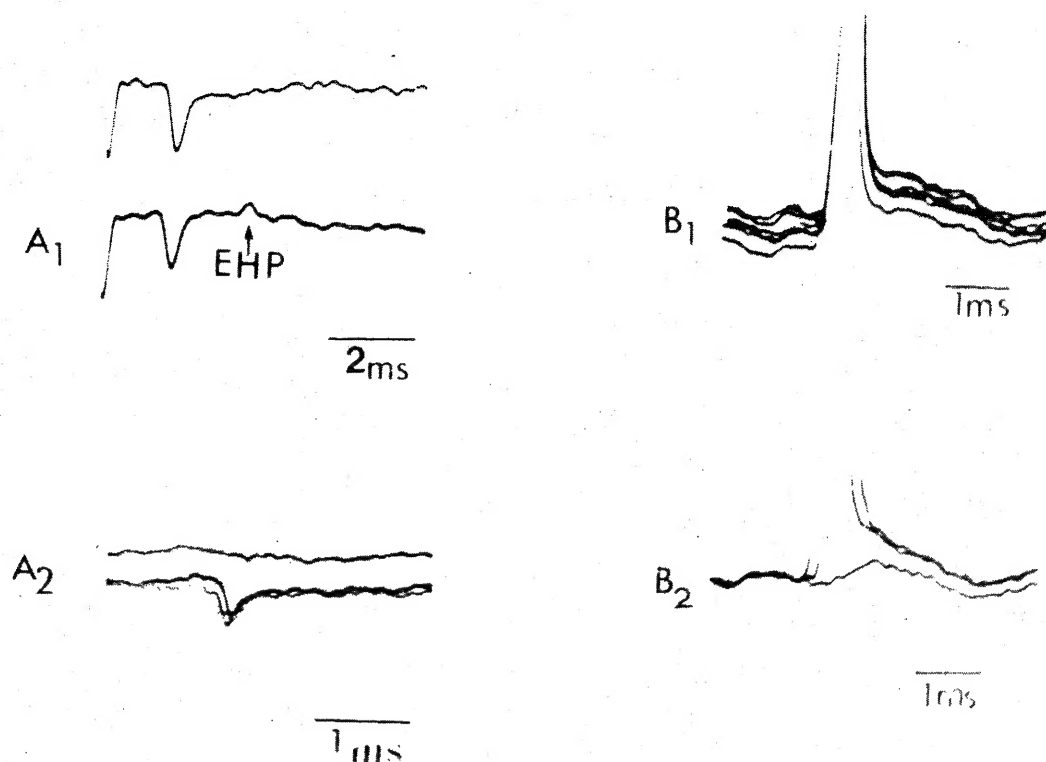


Fig. 5. The antidromic activations of the Mauthner cells stimulating the spinal cord at strengths straddling threshold.

- A1: Antidromic field potential with EHP (The base line is arbitrarily indicated by a horizontal line).
- B1: Intracellular recording of the antidromic activation.
- A2: All-or-none antidromic field potential. Below threshold stimulation (upper trace) shows no response. Increasing stimulating voltage lead to full size potential (lower trace).
- B2: Only a small delay EPSP is present on below threshold antidromic activation. Increasing stimulating voltage giving a fully fledged action potential. This could be from contralateral Mauthner cell activation. Calibration: 10 mV, 1 msec.

is a short latency, all or none negative potential which is similar to those of the other teleosts, e.g. goldfish (Faber and Korn, 1978; Eaton *et al.*, 1981, 1982; Funch and Faber, 1982a), winter flounder (Zottoli, 1981). The short latency fits with the fact that Mauthner axons are the fastest conducting fibers in the spinal cord, with a range of 70–100 m/sec (Faber and Korn, 1978; Zottoli, 1981; Eaton *et al.*, 1981, 1982; Funch and Faber, 1982b; Eaton and Hackett, 1984). Zottoli (1977) showed that goldfish, 11.5 to 14.5 cm in length, have

a latency to antidromic stimulation of caudal spinal cord of 0.91 msec. Furshpan and Furukawa (1962) assumed a value of 100 m/sec for the conduction velocity of 80 m/sec by calculating the delay of two spikes recorded from two electrodes separated by 3 mm. Measurements using a single electrode were 17% larger than those determined using two electrodes (Funch and Faber, 1982a, b). In the present experiments, using a single recording site, conduction velocities were around 100 m/sec measured with a conduction

length of about 20 cm and a mean latency around 1.9 milliseconds. Allowing for error in single electrode studies, likely values are around 85 m/sec.

The extracellular antidromic spike recorded in the region of the axon cap has an all or none characteristic (Fig. 5A2). A discrete hyperpolarization, the extrinsic hyperpolarising potential (EHP), can be recorded from this region (Fig. 5A1), but on intracellular penetration it no longer appeared (Fig. 5B2). This potential is characteristic of the Mauthner axon cap region only and is a valuable aid in identification (Faber and Korn, 1978; Zottoli, 1981). The EHP has a longer latency than the antidromic spike, and can be elicited in the absence of an antidromic response in the Mauthner cell.

The Mauthner cell could be more accurately localized with the aid of an antidromically evoked positive potential, which referred to the EHP (Zottoli, 1981). It arises from certain extracellular potential changes, which passively hyperpolarize the axon cap region. In fact, it is a type of electrical inhibition (Furshpan and Furukawa, 1962; Faber and Korn, 1978). Cod clearly shows this potential with its characteristically long latency relative to the antidromic spike, which appears only in extracellular records, suggesting a common origin to the EHP as in other fish, goldfish and winter flounder (Furukawa and Furshpan, 1962; Faber and Korn, 1978; Zottoli, 1981). Differences in inhibitory synaptic inputs to the axon cap region may be important in the control of Mauthner excitability under conditions of effective sensory stimulation. Zottoli (1978) suggested that the electrical component of the inhibition at least was dependent on the integrity and high electrical resistance of the axon cap and the glial element. This suggests that there may be a functional separation corresponding to the morphological separation into two types of axon caps.

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REFERENCES

- Anthony, P.D. (1981) Visual contrast threshold in the cod, *Gadus morhua* L. *J. Fish Biol.* **19**: 87-103.
- Brown, A.G. and R.E.W. Eyffe (1984) Intracellular staining of mammalian neurones. Academic Press. N.Y. 115 pp.
- Eaton, R.C. (1983) *Is the Mauthner cell a vertebrate command neurone? A neuroethological perspective on an evolving concept.* In: *Advances in Vertebrate Neuroethology* (J.D. Ewert, R. Capranica & D.J. Ingle ed.). Plenum Press. pp. 629-636.
- Eaton, R.C. and J.T. Hackett (1984) *The role of the Mauthner cell in fast-starts involving escape in teleost fishes.* In: *Neural Mechanisms of Startle Behaviour.* (R.C. Eaton ed.) Plenum Press. pp. 213-266.
- Eaton, R.C., R.A. Bombardier and D.L. Meyer (1977) The Mauthner-initiated startle response in teleost fish. *J. Expt. Biol.* **66**: 65-81.
- Eaton, R.C., W.A. Lavender and C.M. Wieland (1981) Identification of Mauthner-initiated response patterns in goldfish: evidence from simultaneous cinematography and electrophysiology. *J. Comp. Physiol.* **144**: 521-531.
- Eaton, R.C., W.A. Lavender and C.M. Wieland (1982) Alternative neural pathways initiate fast-start responses following lesions of the Mauthner neuron in goldfish. *J. Comp. Physiol.* **145**: 485-496.
- Eaton, R.C., J. Nissarov and C.M. Wieland (1984) Differential activation of Mauthner and Non-Mauthner startle circuits in the zebrafish: implications for functional substitution. *J. Comp. Physiol.* **155**: 813-820.
- Faber, D.S. and H. Korn (1978) *Neurobiology of the Mauthner cell.* Raven Press. 279 pp.
- Funch, P.G. and D.S. Faber (1982a) Cable properties of goldfish Mauthner axon. *J. Neurophysiol.* **47**: 1196-1213.

- Funch, P.G. and D.S. Faber (1982b) Action-potential propagation and orthodromic impulses initiation in Mauthner axon. *J. Neurophysiol.* **47**: 1214-1231.
- Furshpan, E.J. and T. Furukawa (1962) Intracellular and Extracellular responses of the several regions of the Mauthner cell of the goldfish. *J. Neurophysiol.* **25**: 732-741.
- Guthrie, D. M. (1980) *Neuroethology: an introduction*. Blackwell Scientific Publications. 221 pp.
- Horner, K. (1980) Structure and function of the codfish ear. Ph.D. thesis, University of Aberdeen, U.K.
- Huang, B.Q. (1986) Visually evoked startle responses in the cod (*Gadus morhua*) Ph.D. thesis, University of Aberdeen, U.K.
- Huang, B.Q. and P.J. Fraser (1989) Flash-evoked c-escape behavior from free swimming codfish (*Gadus morhua*). *Bull. Inst. Zool., Academia Sinica* **28**: 97-106.
- Kimmel, C.B., R.C. Eaton and S.L. Dowell (1980) Decreased fast-start performance of zebrafish larvae lacking Mauthner neurones. *J. Comp. Physiol.* **140**: 343-350.
- Nakajima, Y. (1974) Fine structure of the synaptic endings on the Mauthner cell of the goldfish. *J. Comp. Neuro.* **156**: 375-402.
- Purves, R. D. (1981) *Microelectrode methods for intracellular recording and iontophoresis*. Academic Press, New York. 145 pp.
- Retzlaff, E. (1957) A mechanism for excitation and inhibition of the Mauthner cell in teleost: a histological neurophysiological study. *J. Comp. Neuro.* **107**: 209-225.
- Webb, P.W. (1978) Fast-start performance and body form in 7 species of teleost fish. *J. Exp. Biol.* **74**: 211-226.
- Zottoli, S.J. (1977) Correlation of the startle reflex and Mauthner cell auditory responses in unrestrained goldfish. *J. Exp. Biol.* **66**: 243-253.
- Zottoli, S.J. (1978a) Comparison of Mauthner cell size in teleosts. *J. Comp. Neuro.* **178**: 741-758.
- Zottoli, S.J. (1978b) *Comparative morphology of the Mauthner cell in fish and amphibians*. In: *Neurobiology of the Mauthner Cell* (D. Faber and H. Korn ed.). Raven Press, N.Y.
- Zottoli, S.J. (1981) Electrophysiological and morphological characterization of the winter flounder Mauthner cell. *J. Comp. Physiol.* **143**: 541-553.
- Zottoli, S.J., D.H. Hangen and D.S. Faber (1984) The axon reaction of the goldfish Mauthner cell and factors that influence its morphological variability. *J. Comp. Neuro.* **230**: 497-516.

鱈魚 (*Gadus morhua*) Mauthner 細胞之形態與生理

黃寶貴 Peter J. Fraser

本研究為探討鱈魚 (*Gadus morhua*) 之 Mauthner 細胞之形態與生理特徵，結果顯示該細胞在形態上具有其他硬骨魚 Mauthner cell 之四項特徵：(1) 細胞本體位於第 8 對腦神經之平行位置；(2) 具有顯著的側向樹突及腹向樹突；(3) 具大之軸突；(4) 明顯之軸突鞘。另其有具側腹向之樹突。逆行刺激所記錄之胞外記錄亦具有極短之潛伏期（約 1.9 毫秒）及全或無電位，並符合 Mauthner 細胞生理之其他特徵。